Naturally Occurring Biodegradation as a Remedial Action Option for Soil Contamination

Interim Guidance (Revised)

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NATURALLY OCCURRING BIODEGRADATION
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INTERIM GUIDANCE (Revised)

INTRODUCTION

This document is intended to provide interim guidance on using naturally occurring biodegradation as a remedial action option. The emphasis here is on petroleum hydrocarbon contamination in unsaturated zone soils. While much of what is discussed is applicable to other organic contaminants and to groundwater contamination, these situations involve additional considerations not addressed in this interim guidance.

The first section provides some general background information on naturally occurring biodegradation, how it works, and when it may be appropriate as a remedial action. The second section discusses site characterization and considerations for evaluating the suitability of a site for naturally occurring biodegradation. The third section discusses long-term monitoring of natural biodegradation.

The material discussed in this document is not comprehensive. DNR project managers have the authority to determine specific characterization and monitoring requirements based on site conditions. Please feel free to call Mike Barden at (608) 264-6007 for clarification or elaboration of specific points, or to discuss how naturally occurring biodegradation may relate to specific sites.

BACKGROUND

"Naturally occurring biodegradation" means degradation of organic compounds by indigenous microbes without artificial enhancement. The terms "passive bioremediation" and "intrinsic bioremediation" are also used to describe utilization of naturally occurring biodegradation as a remedial action.

Application of naturally occurring biodegradation as a remediation technique requires that a site be evaluated to ensure site conditions are appropriate and that a monitoring plan be developed. Characterization of a site for evaluation of naturally occurring biodegradation potential should be part of the initial site investigation. Naturally occurring biodegradation is considered to be a remedial action, and its suitability to a given site should be considered during evaluation of possible remedial action options and selection of an overall site remedial action plan.

Situations where naturally occurring biodegradation may be appropriate

Naturally occurring biodegradation may be a suitable remedial action at sites where the contaminants of concern are readily biodegradable, site conditions are favorable, and the time necessary for naturally occurring biodegradation to effect cleanup is reasonable considering the site-specific circumstances. Naturally occurring biodegradation is a <u>long-term</u> remediation option; it may require years or decades to effect adequate clean-up of a site, depending on site conditions. This long time-frame for remediation may be acceptable to the Department so long as the potential impacts from contamination at a site are minimal. This determination will depend on factors such as:

- magnitude, toxicity, and mobility of the contaminants given the geologic and hydrogeologic conditions at the site;
- proximity of human and environmental receptors, especially sensitive human receptors and threatened or endangered species or habitats;
- proximity to private and public water supplies;

- potential use of the aquifer in the vicinity of the site in the future; and
- reliability and enforceability of institutional controls.

In general, naturally occurring biodegradation may be used:

- as a primary remedial action at low- and medium-priority sites where contamination is confined to unsaturated soil and levels of contamination are sufficiently low so that groundwater quality is not threatened;
- as a second phase of remediation after an active remediation process has sufficiently reduced contaminant concentrations in unsaturated soil to levels where contaminant migration to the groundwater that would violate ch. NR 140 is unlikely; or
- as a component of a more comprehensive remediation plan.

Naturally occurring biodegradation may also be appropriate as a remedial action at sites with low-level groundwater contamination. Such situations could include sites where contaminant concentrations are below ch. NR 140 enforcement standards and some sites where contaminant concentrations are slightly above enforcement standards and the potential for contaminant migration and impacts is minimal. However, additional site characterization, beyond that described in this guidance, and evaluation of potential contaminant migration and impacts will be required.

Naturally occurring biodegradation implies that no active measures are taken to amend site conditions. In some cases, where site conditions are not entirely favorable to naturally occurring biodegradation, some minor modification of site conditions may allow bioremediation to be employed. This may still provide a low-cost remediation alternative; however it involves active modification of site conditions and treatability studies may be required to evaluate the potential effectiveness. Examples of such sites could include:

- a site where available nutrients are limiting, where the simple addition of fertilizer to increase the available nutrients may be adequate; or,
- a site where oxygen is limiting, as is likely in clayey tills, where the installation of a low flow-rate soil venting system may be adequate to increase oxygen availability.

Many site modifications require prior DNR consultation and approval. The major exception is air-flow enhancement carried out through installation of a positive- or negative-pressure system; written guidance will be available for such systems. Note also that the use of injection wells (e.g., for introduction of nutrients or microbes) is generally not allowed in Wisconsin (contact the Bureau of Water Supply for specific requirements). Written guidance for reinfiltration/reinjection systems is being prepared.

The biodegradation process

Many indigenous microbes in soil and groundwater are capable of transforming both naturally occurring and artificial hydrocarbon compounds through direct metabolism. The term "biodegradation" may refer to complete mineralization of the organic contaminants to carbon dioxide, water, inorganic compounds, and cell protein, or to transformation of organic contaminants to other organic compounds.

Biodegradation of organic constituents is accomplished by enzymes produced by microorganisms. Since many enzymes are not released by microbial cells, substances to be degraded must generally contact or be transported into the cells. Enzymes are generally specific to the substances they affect, so many types may be required to completely biodegrade organic constituents. Biodegradation of a compound is typically a step-wise process involving a variety of different enzymes and species of organisms. Therefore, in the natural environment, a constituent may not be completely degraded, but only transformed into intermediate product(s) that may be less, equally, or more hazardous than the original (parent) compound, as well as more or less mobile in the environment. Additionally, many hydrocarbon compounds that cannot be utilized as a carbon or energy source by microorganisms can be degraded by enzymes generated by microbes to metabolize other compounds. This process is referred to as cometabolism. The non-growth substrate is typically only incompletely oxidized (transformed) by the microbe involved, but other microbes may utilize by-products of the cometabolic process. Cometabolism may be a prerequisite to the mineralization of many recalcitrant compounds, such as PAH compounds.

Biodegradation is fundamentally an electron transfer process. Biological energy is obtained through the oxidation of reduced materials. Microbial enzymes catalyze the electron transfer. Electrons are removed from organic substrates to capture the energy that is available through the oxidation process. The electrons are moved through respiratory or electron transfer chains (metabolic pathways) composed of a series of compounds to terminal electron acceptors. A large proportion of the microbial population in soil depends upon oxygen as the terminal electron acceptor for metabolism. Loss of oxygen induces a change in the activity and composition of the soil microbial population. Facultative anaerobic organisms (which can use oxygen when it is present or can switch to alternative electron acceptors, such as nitrate and sulfate, in the absence of oxygen) and obligate anaerobic organisms become dominant when oxygen is not available. Biodegradation can occur under both aerobic (oxygen present) and anaerobic (oxygen absent) conditions; aerobic biodegradation is typically more efficient.

A wide variety of organic materials are easily degraded under aerobic conditions. In aerobic metabolism, O_2 is the terminal electron acceptor. When biodegradation follows this pattern, microbial populations quickly adapt and reach high densities. As a result, the rate of biodegradation quickly becomes limited by rate of supply of oxygen or some nutrient, not the inherent microbial capacity to degrade the contaminant. The ultimate products of aerobic metabolism are carbon dioxide and water.

Some organic compounds can also be degraded under anaerobic conditions. When oxygen is absent, nitrate (NO_3^-), sulfate (SO_4^-), ferric iron (Fe^{3+}), manganese (Mn^{3+}, Mn^{4+}), and bicarbonate (HCO_3^-) can serve as terminal electron acceptors, if the microbes have the appropriate enzyme systems. Under anaerobic conditions, the rate of degradation is usually limited by the inherent reaction rate of the active microorganisms; adaptation is slow, requiring months or years, and metabolic activity results in the formation of incompletely oxidized, simple organic substances, such as organic acids, and by-products such as methane or hydrogen gas.

Numerous factors affect the potential for and rate of naturally occurring biodegradation at a given site, such as:

- soil moisture content
- porosity
- soil temperature
- soil pH
- O_2 availability
- redox potential
- presence of suitable microbes
- contaminants present and their concentrations
- availability of nutrients
- presence of other electron acceptors

Because of the dependence on these factors, adequate site characterization is essential for determining the viability of naturally occurring biodegradation as a remediation option at a given site.

SITE CHARACTERIZATION

Characterization of a site for evaluation of naturally occurring biodegradation potential should be part of the initial site investigation. The site characterization discussed here is specific to evaluating naturally occurring biodegradation in unsaturated soils, and is in addition to a site investigation done in accordance with the DNR *Guidance for Conducting Environmental Response Actions*, March, 1992 (PUBL SW-157-92). The characterization for naturally occurring biodegradation potential will involve:

- characterization of the contaminants at a site;

- assessment of physicochemical conditions and presence of appropriate nutrients; and

- in some cases, assessment of microbiological parameters to determine the presence and viability of an appropriate microbial population may be necessary.

Characterization of site heterogeneity and evaluation of potential migration of contamination to groundwater should be included in the site investigation. Results of the site investigation may indicate that the potential for groundwater contamination or the risks from direct contact make the use of naturally occurring biodegradation as a remedial action inappropriate for the site.

The number of samples necessary to adequately characterize a site for naturally occurring biodegradation potential will vary based on the extent of contamination and the heterogeneity of the soils at the site. Sufficient samples should be analyzed to be representative of the distribution of contaminant concentration and affected soil types. At least one sample from outside the zone of contamination should be analyzed to provide a control.

Contaminant Characterization

Contaminants present and their concentrations

Identification of the contaminants at a site is necessary to evaluate whether naturally occurring biodegradation is appropriate as a remediation option. This is important both for evaluation of the potential for migration of contaminants to groundwater and for determining that the compounds present are readily amenable to naturally occurring biodegradation. Contaminant analyses should be conducted in accordance with the *LUST Analytical Guidance*, July, 1993 (PUBL-SW-130 93REV). A complete VOC analysis should be done for at least one sample at all sites, together with GRO and DRO analyses. At sites where diesel, fuel oil, or heavy oil contamination is involved, PAH analysis should also be done.

Degradation of most volatile compounds is inhibited whenever organic vapors are present in high concentrations. This is due to acute toxic effects or to reduced oxygen levels in the soil gas. Acute toxicity to microorganisms is unlikely when total contaminant concentrations in soil are at residual levels of less than several hundred mg/kg GRO or about 1000 mg/kg DRO.

Biodegradability

Most petroleum hydrocarbons are readily biodegradable through aerobic metabolism. Many are also biodegraded by anaerobic metabolism, although at lower rates. In general:

- Water soluble compounds are usually degraded faster than less soluble compounds.
- The n-alkanes, n-alkylaromatics, and aromatic compounds in the C_5 to C_{22} range are

usually readily biodegradable. These compounds comprise a major portion of gasoline, diesel, and fuel oil.

- The n-alkanes, n-alkylaromatics, and aromatic compounds above C_{22} have very low water solubilities which result in slow degradation rates. These compounds are common in heavier oils.
- Condensed or fused aromatic and cycloparaffinic compounds with four or more rings have very low biodegradation rates. These include most PAH compounds.
- The rate of oxidation of straight-chain aliphatic hydrocarbons is inversely correlated to chain length.

The BTEX compounds are typically removed at about the same rate by aerobic metabolism. Under anaerobic metabolism, these aromatic compounds are first oxidized to phenols or organic acids, then transformed to long-chain volatile fatty acids, which are finally metabolized to methane and carbon dioxide. The biodegradability and degradation rates for each of these compounds under anaerobic conditions can vary considerably.

Many chlorinated hydrocarbons are also readily biodegradable through aerobic and/or anaerobic metabolism. However, when significant concentrations of these compounds are present, the application of naturally occurring biodegradation should be considered carefully due to the potential for production of metabolites having greater toxicity than the original contaminant. Knowledge of the applicable microbial metabolic pathways is necessary. For example, the anaerobic metabolism of TCE produces vinyl chloride as a metabolite, which is significantly more toxic than the parent compound. In such situations, naturally occurring biodegradation would not be acceptable.

Environmental Parameters

Characterization of environmental parameters at a site is essential for evaluating naturally occurring biodegradation potential. This involves a determination that the physical and chemical conditions at the site are amenable to naturally occurring biodegradation occurring. The specific parameters that need to be evaluated for a given site must be determined on a site-specific basis. For unsaturated soils, these parameters may include the following, not in necessarily in any order of relative importance:

- soil moisture content
- soil water holding capacity/field capacity
- porosity
- permeability
- bulk density
- soil temperature
- soil pH
- soil water dissolved oxygen
- soil gas oxygen content
- organic matter content (OM)
- total organic carbon (TOC)
- total organic nitrogen (TON)
- redox potential (Eh)
- inorganic nitrogen (as NH_3, NO_2, NO_3)
- soluble phosphorous (o-PO_4)
- "soluble" manganese (Mn^{2+})
- iron (Fe^{3+}, Fe^{2+})
- sulfate (SO_4^{2-})

Some additional parameters may also be important for particular sites or contaminants, such as:

- soluble potassium
- soluble salts
- cation exchange capacity (CEC)
- exchangeable cations
- buffer index
- Na, Ca, Mg, S, B, Cu, Zn

Not all of these parameters need to be measured directly; some can be estimated from measured parameters since they are inter-related. In many cases, an adequate evaluation can be based on only a few of these parameters. However, the additional information is useful and can provide justification for using naturally occurring biodegradation as a remedial action at "marginal" sites. Most of these are standard soil analysis parameters which can be obtained from a single soil analysis. The analyses are typically not expensive, and it is probably more cost-effective to obtain the data initially, rather than have to re-sample later. Some analytical methods for these parameters include American Society for Testing and Materials (ASTM) methods, U.S. EPA methods (SW-846), and those in Klute (1986) and Page *et al* (1982).

Microbial ecologists have identified ranges of critical environmental conditions that affect the activity of soil microorganisms. The following is a brief discussion of site conditions that are important for naturally occurring biodegradation in unsaturated soils, and how they may be evaluated to determine the suitability of a site for using naturally occurring biodegradation as a remedial action. Where possible, ranges of values and/or critical values for specific parameters that are important for evaluating biodegradation potential are provided.

Soil moisture

Soil moisture is essential to biodegradation since the majority of microorganisms live in the water film surrounding soil particles. Soil water serves as the transport medium through which many nutrients and organic constituents diffuse to the microbial cell, and through which metabolic waste products are removed. Soil moisture content also affects the soil aeration status, nature and amount of soluble materials, soil water osmotic pressure, and the pH of the soil.

The degree to which the soil pore space is filled with water affects the exchange of gases through the soil. When soil pores become filled with water, the diffusion of gases through the soil is severely restricted, oxygen is consumed faster than it is replenished in the soil vapor space, the soil becomes anaerobic, and major shifts in microbial metabolic activity occur. (see Oxygen availability below)

Soil moisture content should be in the range of 25-85% of the water holding capacity; a range of 50-80% is optimal for biodegradation. The soil water holding capacity is equivalent to the "field capacity," which is the percentage of water remaining in a soil after it has been saturated and gravitational drainage has ceased. The water holding capacity is not a unique value. It is a function of the matric potential of the soil water and represents a range of soil water contents that are dependent on soil type. The soil moisture content at the water holding capacity generally corresponds to matric potentials in the range of -0.01 to -0.015 MPa (-0.1 to -0.15 bar) in sands and -0.03 to -0.05 MPa (-0.3 to -0.5 bar) in medium- to fine-textured soils.

The matric potential of the soil water, against which microorganisms must work to extract water from the soil, regulates microbial activity. (The soil matric potential is a measure of the energy required to overcome capillary and adsorptive forces.) In general, microbial activity becomes reduced at a matric potential of about -0.1 MPa (-1 bar), but can be carried out at matric potentials from -0.5 MPa (-5 bars) to lower than -1.5 MPa (-15 bars) without much inhibition. This generally corresponds to the range of soil moisture content noted above. The lower limit for all bacterial activity is about -8 MPa (-80 bars). However, a matric potential of -0.01 MPa (-0.1 bar) or greater is considered optimal for microbial activity.

Permeability

Permeability is a measure of the ability of the soil to transmit fluids, such as water or air. It is an important factor for biodegradation because it controls the movement of water, gases, and nutrients through the soil system. In unsaturated soils, the permeability varies with the soil moisture content, and the water permeability (hydraulic conductivity) and air permeability have an inverse relationship.

In general, saturated hydraulic conductivity for the soil should be greater than 10^{-3} cm/sec for optimal biodegradation conditions; values in the 10^{-5}-10^{-3} cm/sec range may be acceptable if other site characteristics are favorable.

Soil temperature

Soil temperature is a controlling factor for the rate of biodegradation. Higher soil temperatures result in higher microbial metabolic activity and higher rates of biodegradation. Biodegradation essentially stops at 0°C. If a viable microbial population is present at a site, then soil temperature must be appropriate for at least part of the year. However, the rate of biodegradation may vary seasonally. Also, microbial metabolic activity can itself increase soil temperature.

A better estimate of rates of naturally occurring biodegradation based on literature values is possible when the temperature is known. Most reported biodegradation rates have been determined at 20°-25°C. Use of these rates is not appropriate for lower soil temperatures, such as in Wisconsin where average soil temperatures at depth are around 10°C. In general, the rate of biodegradation is decreased by one-half for each 10°C decrease in temperature. However, the biodegradation rate for a compound at a given site is controlled by many factors, of which temperature is only one, and such estimates should be used with caution.

Soil pH

Soil pH is an indicator of hydrogen ion activity in the soil. A pH in the range of 5 to 9 is generally acceptable for biodegradation; a pH of 6.5 to 8.5 is generally considered to be appropriate for optimal biodegradation efficiency.

Soil pH also affects the availability of nutrients. The solubility of phosphorous, an important nutrient in biological systems, is maximized at a pH value of 6.5.

Oxygen availability

A large fraction of the microbial population within soil depends on oxygen as the terminal electron acceptor in metabolism, and the rate of aerobic biodegradation is typically limited by the rate at which oxygen is supplied. The major source of oxygen in soil is diffusion from the atmosphere. When soil pores become filled with water, the diffusion of gases through the soil is restricted. Oxygen may be consumed faster than it can be replaced by diffusion from the atmosphere, and the soil may become anaerobic. Clayey soils tend to retain a higher moisture content, which restricts oxygen diffusion. Organic matter may increase microbial activity and deplete available oxygen.

The solubility of oxygen in water is low at best; about 10 mg/l at 25°C. For aerobic metabolism, dissolved oxygen concentrations in the soil moisture of greater than 0.2 mg/l are necessary; oxygen becomes rate-limiting at dissolved oxygen concentrations below about 1 mg/l. Soil water dissolved oxygen content is difficult to measure directly, however it can be estimated from O_2 concentrations measured in soil gas.

Oxygen levels in soil gas should be at least 2-5% in order to avoid oxygen limitation of aerobic microbial activity. A minimum air-filled porosity of 10% is necessary to allow for adequate oxygen diffusion in the soil gas. The air-filled porosity can be calculated by subtracting the volumetric water content from the total porosity. Total soil porosity can be measured directly, or be estimated from bulk density where:

$$\text{porosity} = 1 - (\text{dry bulk density} / \text{average particle density}).$$

A value for the average particle density of soil solids that is commonly used is 2.65 g/cm^3.

Redox potential

The oxidation-reduction potential (redox potential) of a soil provides a measurement of the electron density of the system. Biological energy is obtained from the oxidation of reduced materials. In aerobic processes, O_2 acts as the terminal electron acceptor. As a system becomes reduced, O_2 is depleted, and other substances are used as terminal electron acceptors resulting in a progressive increase in electron density (negative potential).

A low electron density (*Eh* greater than 50 millivolts) indicates oxidizing, aerobic conditions; high electron density (*Eh* less than 50 millivolts) indicates reducing, anaerobic conditions. High positive *Eh* values (400 mV to 800 mV) indicate well aerated conditions that are optimal for aerobic biodegradation. Values for *Eh* of 100 mV to 400 mV generally indicate reduced oxygen conditions, but are acceptable for aerobic biodegradation. Measurements of redox potential in soil are typically made using a platinum electrode and a reference electrode. These are inserted in the soil and the potential difference is measured.

Soil color can generally provide a qualitative indication of redox conditions. Uniform red, yellow, or brown color indicates oxidizing conditions. Uniform gray or blue color indicates reducing conditions. Mottled soils indicate variable conditions.

Availability of nutrients

Microbial metabolism and growth is dependent upon the availability of essential nutrients in: 1) a usable form, 2) appropriate concentrations, and 3) proper ratios. Carbon (C), nitrogen (N), and phosphorous (P) are essential nutrients. Biodegradable organic compounds provide a carbon source, and total organic carbon (TOC) is a measure of the total carbon, or "food," that microbes may utilize for energy and growth. All organic chemicals present in the soil are included in the TOC measurement. Nitrogen in the form of organic nitrogen, ammonia (NH_3), nitrite (NO_2), and nitrate (NO_3), and phosphorous in the form of soluble or reactive phosphorous (o-PO_4, or ortho-phosphate) are suitable to microbes for growth. Nitrate can also provide energy to microbes when oxygen levels are low.

The amount of nitrogen in decomposing organic matter is important in controlling the rate of contaminant degradation by microorganisms. Total organic nitrogen (TON) is a measure of this and total organic matter which contains greater than 1.5% to 1.7% nitrogen is probably adequate to meet the microbial nitrogen requirements during contaminant metabolism. TON is measured as kjeldahl N less inorganic N. A C:N ratio of less than 20-25 should lead to mineralization (excess N present); a C:N ratio of greater than 35-40 generally indicates inadequate nitrogen, which could limit biodegradation due to depletion of mineralized nitrogen resulting in nitrogen starvation. Similarly, immobilization of phosphorous can occur when the C:P ratio is greater than 120:1. A suggested C:N:P ratio of 100:10:1 is considered optimal. Ratios should be used with caution, however, since they do not indicate the availability of the carbon, nitrogen, or phosphorous to microorganisms.

Other nutrients (K, Ca, Mg, S, etc.) are typically found in adequate supply for metabolic needs in most soils. However, high concentrations of calcium and magnesium may precipitate phosphates, and will reduce the amount available for microbial metabolism. High levels of chlorides may inhibit microbial activity.

Presence of other electron acceptors

Oxygen is the primary electron acceptor for aerobic biodegradation processes. However, in situations where oxygen levels are low, other terminal electron acceptors may be utilized for microbial metabolism. Nitrate (NO_3^-), iron (Fe^{3+}), manganese (Mn^{3+}, Mn^{4+}), and sulfate (SO_4^{2-}) can act as electron acceptors if the organisms have the appropriate enzyme systems. Sulfate (SO_4^{2-}) is utilized by microbes when the supply of oxygen or nitrate is low. Microbes can reduce the sulfate molecule to provide energy for metabolism (biodegradation).

Microbiological Characterization

The presence of suitable microbes for degrading the organic contaminants at a site is critical for implementation of naturally occurring biodegradation as a remedial action. Petroleum hydrocarbon-degrading microbes are widespread in the subsurface; in most cases they can be assumed to be present. However, some site conditions, such as marginal environmental conditions or high concentrations of contaminants or organic vapors, make it necessary to demonstrate that a viable microbial population is present. In these cases, microbiological characterization should be conducted to ensure that the site has a viable community of microorganisms to accomplish biodegradation of the organic constituents present.

Enumeration studies

Microbial enumeration studies employ plate counts to determine relative numbers of total aerobic heterotrophs and total hydrocarbon degraders. These studies are useful as qualitative measures for comparison of "clean" versus contaminated areas of a site. These laboratory studies can provide evidence that the necessary microorganisms are present at a site and that metabolic adaptation has occurred. However, it is difficult to relate these studies to biodegradation potential directly since laboratory conditions typically do not replicate site conditions. Enumeration studies are probably most useful for comparison of the areas of highest contamination, where aerobic microbial populations may be significantly reduced, to uncontaminated areas.

Soil samples for microbial enumeration studies must be collected aseptically, using a sterilized sampling apparatus to prevent contamination by nonindigenous microbes. Several specialized sampling devices have been developed; however, most current sampling involves dissection of core samples. The outer few centimeters and the top and bottom of the core sample are removed with an alcohol-sterilized spatula, and only the center portion of the core is used. Samples should be cooled to 4°C, but not frozen, for transport to the laboratory.

Respirometry

An indirect method for determining the presence of a viable microbial community is the use of soil respirometry. This method also has the advantage of providing an indication that *in situ* biodegradation is occurring at a site. Soil respirometry measures O_2 depletion/CO_2 production in the soil and can provide a measure of biological activity. In general, a given amount of oxygen is provided to the soil and the decrease in O_2 concentration and/or increase in CO_2 concentration are monitored over time. Soil respirometry measurements from contaminated areas must be compared with background measurements from outside the zone of contamination at the site. Increased O_2 depletion/CO_2 production in the contaminated area relative to the background rate indicates that aerobic biodegradation is occurring.

Both *in situ* and laboratory methods are available; *in situ* methods probably better represent field conditions, but possible chemical interferences with measurements, such as inorganic sources of CO_2, must be considered.

Minimum Site Characterization Requirements for Naturally Occurring Biodegradation Projects

Determining whether a site is suitable for naturally occurring biodegradation ultimately comes down to professional judgement. In the future, the Department and consultants will benefit from greater experience with naturally occurring biodegradation results on actual sites. However, until more site data become available, the suitability of a site with unsaturated soil contamination for naturally occurring biodegradation should be based on the following minimum guidelines:

- Contaminant concentrations: Petroleum hydrocarbons only; GRO less than 500 ppm; DRO less than 1000 ppm.
- Soil moisture content: 25 - 85% of "field capacity."
- Oxygen: Minimum air-filled pore space of 10%.
- Permeability: Saturated hydraulic conductivity (for unsaturated zone soil) greater than 1×10^{-5} cm/sec.
- pH: 5.5 - 8.5.
- Redox potential: Greater than 100 mV. Soil color: red, yellow, or brown.
- Nutrients: TON greater than 1.5 percent of OM; C:N ratio less than 40; C:P ratio less than 120.

Additionally, for marginal sites, a consultant should demonstrate the presence of a viable microbial community through an enumeration study or through soil respirometry. Other site characterization evidence evaluated on a case-by-case basis may also indicate that a site falling outside the above guidelines is suitable for naturally occurring biodegradation.

Finally, note that where a site does not meet the minimum criteria, the consultant may propose modifying the site conditions to promote biodegradation. However, such a remedy no longer qualifies as "natural" biodegradation.

Documentation Requirements for Site Characterization and Evaluation

The site characterization and evaluation should be described in the site investigation report. This should include:

- the parameters sampled and the values obtained;

- methods of analysis used (cite reference or describe) and rationale for selection, if appropriate;
- any calculated/estimated values for parameters that were not measured directly and the estimation method(s) used; and
- a discussion of the results in terms of the parameters and considerations described above.

The evaluation should include consideration of potential limiting factors, such as oxygen availability and available nutrient levels. The potential for naturally occurring biodegradation to adequately remediate a site and a monitoring plan should be discussed in the analysis of remedial action options.

MONITORING REQUIREMENTS

If the results of the site characterization are favorable and naturally occurring biodegradation is proposed as a remedial action, a monitoring plan must be developed and implemented in order to evaluate the progress and effectiveness of naturally occurring biodegradation at a site. Monitoring serves several purposes:

- it provides an indication that contaminant concentrations are decreasing over time;
- it insures that the decrease in concentration is due to degradation and not migration of the contaminants;
- it can provide information regarding degradation rates; and
- it can provide data regarding the nature of biodegradation at the site.

From the standpoint of evaluating remediation effectiveness, the monitoring need not distinguish between biodegradation and abiotic degradation or loss of contaminants (such as volatilization).

Monitoring Plan

A variety of approaches and techniques are available for monitoring naturally occurring biodegradation and there is no set standard. A combination of approaches and/or techniques will likely be appropriate at most sites. The monitoring plan must be developed to address the nature of the contaminants and physical conditions at the site.

In cases where contamination is confined to the unsaturated zone, monitoring of uncontaminated soil and/or groundwater below the contaminated soil must be employed to ensure that measured loss of contaminants are not due to migration. If unacceptable levels of contaminant leaching are occurring, a contingency plan to address the situation and prevent significant groundwater contamination must be applied. An unacceptable level of contaminant leaching is indicated by an increase in contaminant concentration below the treatment zone or in groundwater that could potentially result in exceeding a groundwater enforcement standard.

The monitoring plan should be included in the analysis of remedial action options. It must include:

- a description of the monitoring approach(es) and technique(s) to be used;
- a description of the sampling plan;
- the analytes to be sampled; and
- the analytical methods to be used.

At a minimum, sampling should be done quarterly (every three months) for the first year after initiation of the monitoring program, and once annually thereafter, unless otherwise determined by the DNR project manager. The initial quarterly sampling is intended to provide an indication of the seasonal variation in monitoring results, which can be useful for interpreting the subsequent annual monitoring results. The site characterization for nutrient parameters (C, N, P) should be repeated at the end of the fifth year to confirm that adequate nutrients remain available and have not become limiting over time.

Confirmatory borings should be performed at a site when DNR review is requested, unless the DNR project manager determines otherwise.

Monitoring Approaches

A variety of approaches to monitoring biodegradation are available. Those discussed here are generally the most appropriate for most sites. Alternative approaches may be suitable for some sites and contaminants, and an overview of these can be found in Madsen (1991).

<u>Change in concentrations of original contaminants</u>

Degradation of most organic compounds in soil systems may be described by monitoring their disappearance in the soil over time. While this is the most straight-forward approach, it must be verified that any decreases in concentrations are <u>not</u> due to migration of the contaminants.

For soil and water samples, the analytes to be sampled for will depend on the composition of the discharge at the site. Laboratory analytical results from the site investigation may be used to help define the analytes to be examined during long-term monitoring. These can include GRO, DRO, TRPH, PVOC, or VOC analyses, or a combination of these. VOC analytes may be restricted to the PVOC compounds <u>and</u> any other VOC compounds detected in the site investigation. Confirmatory samples at the five-year review stage must include GRO and DRO analyses, as well as VOC, PVOC, and/or PAH analyses as appropriate for the contaminants detected during the site investigation.

Soil gas samples should be analyzed for total organic vapors as well as PVOC compounds.

<u>Change in concentrations of co-reactants</u>

Changes in concentrations of various nutrients (PO_4, NH_4, NO_2/NO_3), electron acceptors (O_2, NO_3, $Fe^{3+, 2+}$, $Mn^{4+, 3+, 2+}$, SO_4), and reaction by-products (CO_2, CH_4, N_2) can potentially provide information on the type and progress of biodegradation. These changes must be compared to those in equivalent samples from outside the area of contamination to provide a control. In monitoring naturally occurring biodegradation, realistic and unbiased control (background) data are required so that valid and defensible comparisons can be made between measured values in contaminated and background conditions.

Changes in physical and physicochemical properties, appropriate to the media being sampled, can be measured as well. These may include soil moisture content, soil pH, redox potential, and temperature. Changes in these parameters can provide information for interpreting the other monitoring results.

These measurements can provide an indication that biodegradation is occurring at a site, and also provide information regarding how it is progressing. This information is most appropriate for "marginal" sites, where information on what is actually occurring may support continued use of naturally occurring biodegradation as remedial action rather than a more costly remediation alternative.

Monitoring Techniques

A variety of monitoring methods are available for various media in the vadose zone and groundwater. No one method is appropriate for all sites; a combination of methods for different media will probably be necessary at most sites. Detailed reviews of the application and limitations of various methods can be found in the attached reference list. Written guidance on specific vadose-zone monitoring techniques appropriate for monitoring naturally occurring biodegradation is being prepared. The following is an overview of potentially applicable monitoring techniques.

<u>Soil gas monitoring</u>

Soil gas monitoring can provide information on changes in contaminant concentrations over time and on the type and level of biodegradation. Changes in VOC levels with time can be used to estimate contaminant removal by either biological or physical mechanisms. Additional information regarding microbial activity can be obtained by monitoring levels of oxygen (O_2), carbon dioxide (CO_2), nitrogen (N_2), and methane (CH_4). Accumulation or depletion of these gases, relative to background levels in the subsurface, may indicate aerobic (O_2, CO_2) or anaerobic (N_2, CH_4) microbial activity.

Soil gas monitoring of contaminant concentrations is most effective for low molecular weight petroleum hydrocarbons with high vapor pressures and low aqueous solubilities. Compounds with boiling points greater than 150°C and vapor pressures less than 10 mm Hg at 20°C (e.g., PAHs) probably will not be present in the vapor phase in sufficient quantities to be detected.

Soil gas monitoring can be done with soil gas surveys or "permanent" soil gas monitoring points. Multi-level sampling should be used in all cases so as to better characterize conditions and the progress of biodegradation at the site.

> <u>Replicate soil gas surveys</u> - Soil gas surveys using probes can be done periodically. These must be based on a statistically valid sampling plan in order to provide for valid comparisons between sampling events.
>
> <u>"Permanent" soil gas monitoring points</u> - The installation of small, narrowly screened, soil gas monitoring points can allow monitoring of specific local soil conditions over time.

<u>Soil borings</u>

Soil samples from soil borings can be used to determine the progress of biodegradation as well as to measure vertical migration of contaminants. Soil core sampling within the zone of contamination can be used to determine decline in contaminant concentrations over time. It can also be used to determine reduction or transformation of co-reactants. Soil core sampling below the zone of contamination can be used to determine whether significant concentrations of contaminants have migrated toward the water table. Soil core sampling is probably more useful at sites with contamination from diesel and heavier oils, where soil gas monitoring is less effective, and where nutrient levels are being monitored.

<u>Soil pore water monitoring</u>

Soil pore water sampling can provide information on contaminant concentrations and soil conditions. Pore water sampling can also be used to detect rapid pulses of dissolved contaminants that move through the unsaturated zone. Heavy precipitation and snow-melt events are often responsible for such pulses, and sampling periods should be scheduled for such events. Soil pore liquid sampling can provide a much more accurate measurement of contaminant migration to the groundwater than does groundwater monitoring alone. Analysis of soil pore liquid can also provide an early warning signal for contaminant migration prior to significant groundwater contamination occurring.

Since water in the unsaturated zone is held under negative pressure, wells can not be used to collect soil pore water. Rather, a variety of lysimeters and pore-water samplers can be used. Detailed reviews of the application and limitations of these devices can be found in Wilson (1990) and other references in the attached reference list.

Groundwater monitoring

Groundwater monitoring is the least useful method of monitoring naturally occurring biodegradation in unsaturated soil, since it provides no information on the progress of biodegradation in the unsaturated zone. Therefore, it should be used in combination with other monitoring techniques.

Groundwater monitoring can be utilized to detect contaminants leaching from unsaturated soil and is probably most appropriate at sites where the base of the zone of contamination is within a few meters of the water table. It will indicate whether groundwater contamination has occurred, but only after the fact. Groundwater monitoring is necessary at sites with potential for migration of contaminants to groundwater or where groundwater contamination is present.

Groundwater monitoring can be done with monitoring wells or with driven probes (e.g., Hydropunch™). Monitoring wells are necessary at sites where groundwater contamination is present. Driven probe samples can be used as a screening method at sites where contamination is initially confined to unsaturated soils in order to determine whether groundwater contamination has occurred.

Reporting and Documentation Requirements for Biodegradation Monitoring

The responsible party (RP) should make arrangements for appropriate monitoring of the site and for timely submittal of reports to WDNR. Reports of monitoring results should be submitted to WDNR annually (results of the quarterly sampling for the first year should be included in a single submittal), unless otherwise determined by the DNR project manager[1]. However, if significant migration of contaminants that could potentially result in exceeding a groundwater enforcement standard is detected at any point, including during the first year of monitoring, WDNR must be notified and a report submitted **immediately**.

Annual submittals need not be in report format, but should include monitoring results in tabular and graph form and a brief discussion of the data and site status. The table and graph of monitoring data in each submittal should include the current monitoring results and all previous results, so as to provide a concise summary of the monitoring program. Sample chain-of-custody forms and laboratory analytical reports should not be included in the annual submittals, unless otherwise determined by the DNR project manager.

Due to the long time-frame involved with naturally occurring biodegradation, it is unlikely that contaminant concentrations will be sufficiently reduced to allow consideration of a site for closeout in less than five years. Therefore, it is recommended that DNR review monitoring results at intervals of not less than five (5) years after initiation of long-term monitoring, at the request of the RP. This review will evaluate the sampling results and determine whether the monitoring program should be continued or

[1] Monitoring summaries should be submitted in duplicate. One copy should be sent to the appropriate district office and one copy sent to the central office, attn: Mike Barden, Wisconsin DNR, SW/3, P.O. Box 7921, Madison, WI 53707.

whether the site can be closed out.

When DNR review is requested, a report should be submitted that describes and evaluates the site status and the monitoring results. This report should include:

- a re-evaluation of the site's biodegradation potential, including a summary and comparison of the initial and confirmatory analysis of nutrient levels;
- a table and graph of all current and previous monitoring results;
- a summary and discussion of all current and previous monitoring results, including an analysis of degradation rates;
- recommendations for further action;
- all sample chain-of-custody forms and laboratory analytical reports, arranged in chronological order; and
- any other data or information specified by DNR in the approval of the remedial action.

The Department will not consider the close-out of a site using naturally occurring biodegradation as a remedial action if sufficient monitoring is not conducted and adequately documented.

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